increases evoked in response to IP_3, released locally in two parts of the cell, did not annihilate but approximately doubledin amplitude. This result suggests that the $[Ca^{2+}]_c$ increase, generated by local release of IP_3, had not regeneratively propagated but diffused passively from the release site. Notwithstanding, Ca^{2+} was required for IP_3-mediated wave progression to occur. Increasing the Ca^{2+} buffer capacity in a small (2 μm) restricted region of the cell immediately in front of a carbachol-evoked Ca^{2+} wave, by photolyzing the caged Ca^{2+} buffer diazo-2, halted progression at the site of photolysis. Failure of local increases in IP_3 to evoke waves appears to arise from the restricted nature of the IP_3 increase to small areas within the cell. When IP_3 was elevated throughout a localized increase in Ca^{2+} propagated as a wave. Together, these results suggest that waves initiate over a relatively large length of the cell and both IP_3 and Ca^{2+} are required for active propagation of the wave-front to occur.

1534-Pos

Increased Calcium Response to Depolarization in Voltage Clamped Skeletal Muscle Cells of a Transgenic Model of Amyotrophic Lateral Sclerosis

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Mitochondrial Ca uptake is believed to help regulate mitochondrial metabolism and synthesis of ATP to meet the demands of muscle contraction. Whether mitochondrial Ca uptake modifies Ca signaling during EC-coupling remains an open question. While studies show that mitochondria in skeletal muscle may take up Ca during contraction, it is not known whether altered mitochondrial Ca uptake can play a role in pathophysiological conditions. Our study on ALS mouse model G93A shows that ALS muscle fibers display defective mitochondria with loss of their inner membrane potential in fiber segments. The finding of localized mitochondrial defects in ALS fibers presents a unique opportunity to test whether changes in mitochondrial function can affect intracellular Ca signaling, as Ca release activity can be compared in regions with or without depolarized mitochondria in the same muscle fiber. By loading muscle fibers with TMRE (a probe of mitochondrial membrane potential) and fluo-4 (a Ca indicator) we characterized simultaneously mitochondrial function and Ca release activity in living muscle fibers. The fiber segment with depolarized mitochondria shows greater osmotic stress-induced Ca release activity. Abolishing mitochondrial inner membrane potential by FCCP or blocking mitochondrial uniporter by Ru360 exacerbates the osmotic stress-induced hyperactive Ca release. Furthermore, we evaluated the voltage-induced Ca transient by patch-clamping ALS fibers and found that fiber segments with depolarized mitochondria displayed 5~25% greater Ca transients. Our study constitutes a direct demonstration of the importance of mitochondria in shaping cytosolic Ca signaling in skeletal muscle. Malfunction of mitochondrial Ca uptake may play an important role in muscle degeneration of ALS. Supported by MDA/NIH.

1535-Pos

CICR and Calcium-Dependent Inactivation, Quantified Through the Response to Artificial Ca Sparks in Single Muscle Cells

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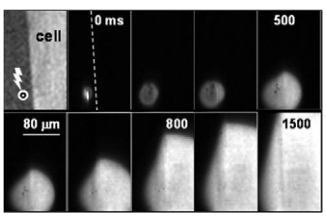
Local calcium stimuli (artificial sparks) generated by 2-photon breakdown of the cage NDBF-EGTA were applied to evoke Ca release from the SR in single skeletal or cardiac muscle cells undergoing fast Ca imaging with the low affinity dye fluo 4FF. The figure shows selected sequential images of the Ca transient generated by a frog skeletal muscle fiber with permeabilized plasmalemma, in response to a spark (elicited *outside* the fiber to avoid photodamage). Two types of responses were observed: (i) an all-or-none wave -shown- that propagates over the entire cell and (ii) graded responses, which fail to propagate. Release analysis (Ríos, JGP 1999; Figueroa, this meeting) separates SR release from simple diffusion of photo-released Ca into cells. The technique yields a sensitive measure of threshold $[Ca^{2+}]$ for release activation, which in the example (0.3 mM $[Mg^{2+}]_{cyto}$) was 1 μM, and can monitor inactivation by combining multiple stimuli. Modeling of these responses aims at describing quantitatively the properties of activation, as well as the roles of inactivation and depletion in the control of Ca release. Other details and acknowledgments are presented elsewhere (Figueroa, this meeting.)



1536-Pos

Flux in Artificial Ca Sparks Generated by 2-Photon Release from a Novel Cage Confocally Imaged at Microsecond Resolution

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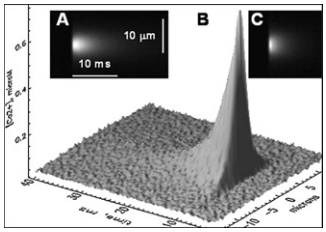
Control of calcium signaling in striated muscle relies on concurrent actions of calcium ions to promote and inhibit release channel opening. To understand these actions we developed artificial Ca sparks generated by 2-photon (2P) release from NDBF-EGTA (Momotake, Nature Methods 2006) as quantifiable local stimuli. A ''Dual Scanner'' (Zeiss) delivers IR laser flashes through a LSM 510 scanner, while rapidly imaging fluorescence of a $[Ca^{2+}]$ monitor via a slit scanner (5-LIVE; *ca* 100 μs/line). Ca sparks of 0.1 to 10 μM (A, B) are elicited in a droplet after microseconds of 2P irradiation at 720 nm and imaged with the low affinity dye fluo 4FF. Reaction-diffusion analysis (Ríos, JGP 1999) yields the flux of Ca photorelease (C). This flux, which initially reaches several hundred mM/s, decays with τ of 2-3 ms. The technique is used to measure physico-chemical properties of calcium ligands, including bio-sensors. Applied inside muscle fibers (Figueroa, this meeting) it serves to quantitatively characterize calcium control in cells.



Instrument purchased with a S10 NCRR award and Hasterlik Family matching funds. Supported by NIAMS, NHLBI/NIH and MDA.

1537-Pos

Effects of High [BAPTA] Inside Mouse Muscle Fibers Reveal a Role of Calcium in the Termination of Voltage-Operated Calcium Release from the SR

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Striated muscles have a termination mechanism that causes the flux of Ca release from the SR, whether activated by an action potential or a voltage pulse, to rapidly cease after an early peak. This mechanism is viewed as a fundamental property that insures stability and ''gradedness'' in the control of Ca signals. To probe this termination, the evolution of Ca release flux was derived from cytosolic Ca transients elicited by SR-depleting depolarizations of long duration, in voltage-clamped cells of mouse FDB muscle. In the presence of 5 mM of the Ca buffer BAPTA, the release flux underwent major changes compared with a 10 mM [EGTA] ''reference'' situation (studied by Royer, J Physiol 2008). Ca release reached an early peak and then decayed to a sustained phase that was higher and briefer than in reference, often including a second rise or ''hump''. In BAPTA, measurable release only lasted 100 ms or less. Its time integral -which measures the SR Ca content releasable by depolarization_was on average 1.4 mM (n=12 cells), compared with 2.1 mM for 18 cells in reference.

An increase in flux with conserved releasable content indicates that BAPTA promotes flux and hastens emptying of the SR without greatly changing its storage properties (including lumenal $[Ca^{2+}]$). A greater Ca flux driven by a similar $[Ca^{2+}]$ gradient requires greater and/or more sustained channel openness. These observations suggest the presence of a release channel-inhibiting mechanism (CDI) mediated by binding of cytosolic Ca^{2+} to open or closed channels, a mechanism more susceptible to interference by BAPTA than the slower-reacting EGTA. Work funded by NIAMS/NIH and an MDA grant to Dr. Jingsong Zhou, who we thank for continued support.

1538-Pos

D4cpv-Casq1. A Novel Approach for Targeting Biosensors Yields Detailed Dynamic Imaging of Calcium Concentration Inside the Sarcoplasmic Reticulum of Living Cells

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Dynamic imaging of $[Ca^{2+}]$ inside the SR of skeletal muscle is hampered by the limited sensitivity of available ratiometric biosensors (Rudolf, JCB 2006) and faces difficulties of calibration when using non-ratiometric dyes (Kabbara and Allen, J Physiol 2001). Impressed by the apparently perfect targeting of